In vitro Establishment and Browning Control of Cinnamon Nodal Explants from Field-Grown Plants (*Cinnamomumm verum*: Sri Gamunu and Sri Vijaya)

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Abstract
Cinnamon is cross pollinated species and wide variability has been observed in yield, quality of produce and oil content and other morphological characteristics. Cinnamon is still exclusively seed propagated. However seeds are highly recalcitrant and therefore, viability diminishes rapidly creating problems for bulking and transporting of seeds for large scale production of plant material. Through consistent efforts, a series of elite cinnamon lines have been identified by cinnamon research station with some superior lines which produce high yields of better quality bark, bark oil and leaf oil. Vegetative propagation techniques like rooted stem cuttings have been reported to be used for experimental purposes but has not been applied at commercial nursery scale. Therefore, there is an urgent need to develop rapid and reliable methods of mass propagation for cinnamon to meet the demand for high quality planting material while conserving their genetic superiority Therefore, this study is proposed to develop a reliable *in vitro* cloning protocol for the commercial scale production of planting material in order to reap the best use of these selected elite cinnamon lines for local cinnamon industry. As the first step, the objective of present study was to control of browning and contaminations in establishing of nodal explants which is crucial for *in vitro* cloning of cinnamon. Experiments were conducted to find out the best surface sterilization process and control of browning in established cultures. Results revealed that 25% Clorox for 10 minutes followed by shaking in ethanol for 30 seconds gave highest non contamination (60%) in nodal explants. Correct maturity stage played a major role in controlling browning along with anti-oxidant or absorbent in the establishment medium. Five weeks after bud emergence (stage 6) were identified as the correct maturity stage for 100% non-brown cultures with ascorbic acid (0.15g L^{-1}) or activated charcoal (1g L^{-1}). Established cultures were inoculated in hormone medium for shoot multiplication.

Keywords: Browning control, Contamination, *In vitro* establishment, Surface sterilization,
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Introduction

Cinnamon (*Cinnamomum verum* J Presl, 2n = 24) is native to Sri Lanka and reported among the earliest known spices used by mankind. Cinnamon belongs to Lauraceae family and to the genus *Cinnamomum* which includes several other important spice and aromatic plant species (Purseglove, 1977). Sri Lanka is the largest exporter of cinnamon (area under cultivation nearly 28, 230 ha) and contributes 60-70% of world true cinnamon trade signifying its importance to local economy.

Cinnamon is a cross pollinated species and wide variability has been observed in yield (Krisnamoorthy *et al.*, 1992), quality of produce and oil content and other morphological characteristics. A high degree of genetic diversity (89% polymorphism) has been reported by Sandigawad and Patil, (2011) in C *verum* (= C *zelanicum*) populations which could probably be attributed to open cross pollination habit of this species and long distance seed dispersal by birds. Even though, Sri Lankan grown cinnamon has a special appeal in world cinnamon trade as a result of its organoleptic properties, continued seed propagation can widen the variability further resulting in low quality bark and oil which can negatively affect the unrivaled position of Sri Lankan cinnamon.

Cinnamon is still exclusively seed propagated. However, seeds are highly recalcitrant and therefore, viability diminishes rapidly creating problems for bulking and transporting of seeds for large scale production of plant material. Through consistent efforts a series of elite cinnamon lines have been identified by cinnamon research station with some superior lines which produce high yields of better quality bark, bark oil and leaf oil. However, safeguarding this genetic superiority of selected elite lines is a challenge through traditional seed propagation (Ranatunga *et al.*, 2004). Vegetative propagation techniques like rooted stem cuttings have been reported for experimental purposes but has not been applied at commercial nursery scale. Therefore, there is an urgent need to develop rapid and reliable methods of mass propagation for cinnamon to meet the demand for high quality planting material while conserving their genetic superiority.

Therefore, this study is proposed to develop a reliable *In Vitro* cloning protocol for the commercial scale production of planting material in order to reap the best use of these selected elite cinnamon lines for local cinnamon industry. As the first step, this study was conducted with the objective of controlling browning and contaminationsin establishing of nodal explants which is crucial for *in vitro* cloning of cinnamon.

Materials and Methods

Maintaining mother bushes

Approximately 1 year old, plants generated from cuttings, were used as sources of explants. Mother bushes were pruned and sprayed with BAP to generate new juvenile vegetative shoots which are going to be used to obtain explants. Nodal segments (20 mm length) with one axillary bud, excised from branches were used as ex-plant sources for culture initiation.

Mother plant bushes were maintained to minimize systemic infections. Plants were maintained under standardized nutrient and pesticide treatments, achieved by weekly sprays of solutions of systemic (Benomyl™ 1.0 g L^{-1}) and contact fungicides (Captan™ 2.0 g L^{-1}), Hybrid insctar™ (1ml L^{-1}) and fertilizer biweekly with Vigor express™ (2 mlL^{-1}).

Surface-sterilization of explants

Initial studies were carried out to find out the optimum surface sterilization process. Different concentrations of Chlorox: 15%, 20% and 25% were used for surface sterilization for exposure durations of 15, 20 and 25 minutes respectively followed by 70% ethanol for 30 seconds. Explants were kept under running tap water for 30 minutes followed by dipping in 1mll^{-1}Daconil solution for 2 ½ hours. Then explants were exposed to Clorox solution with 2 drops of Tween 20 in aseptic conditions.

Establishment of sterilized explants

Murashige and Skoog (MS) medium and Woody Plant Medium (WPM) were tested as establishment media. The pH was adjusted to 5.7±0.1 with NaOH or HCl (0.1 N). The culture media were distributed in aliquots of 25 mL in glass flasks and autoclaved for 15 minutes (121ºC).

Browning control of ex-plants

Nodal segments were used as initial ex- plants and were collected from mother plants maintained in the field. Collected ex-plants were directly dipped into 100 mgl^{-1}ascorbic acid solution. Then the explants were kept under running tap water for 30 minutes followed by dipping in 1ml L^{-1}Daconil™ solution for 2 ½ hours.Ex-plants were surface sterilized with 20% NaOCl for 20 minutes. Woody Plant Medium (WPM) and Murashige and Skoog (MS) basalmedium incorporated with activated charcoalandascorbic acidin different concentrations (as listed below) were used for culture establishment. Applied treatments were listed as 1g L^{-1} Activated charcoal + WPM (T1), 0.15g L^{-1} Ascorbic acid+ WPM (T2), 1g L^{-1} Activated charcoal + MS (T3) and 0.15gL^{-1} Ascorbic acid + MS (T4).

Marks were allocated according to the scale given below to evaluate the relationship between the maturity stage and the percentage of browning.

Sign	Marks
_	9
+	7
++	5
+++	3
++++	1

_ No browning

\+ Browning

(Increase browning appearance with the number of plus marks)

Identify the correct maturity stage to control browning

In preliminary studies it was observed that maturity stage of the ex-plants also plays a major role in controlling browning. Therefore, different maturity stages after the bud emergence were also tested. Six stages were identified for this experiment as shown in the Figure 2. Browning was observed according to the scale given above. MS and WPM media incorporation of activated charcoal and ascorbic acid (1g L^{-1} Activated charcoal + WPM (T1), 0.15g L^{-1} Ascorbic acid+ WPM (T2), 1g L^{-1} Activated charcoal + MS (T3) and 0.15g L^{-1} Ascorbic acid + MS (T4) were used for the study. All experiments were applied separately to both elite lines and were arranged as Completely Randomized Design and mean separation was done with Duncan's Multiple Range Test. Non-parametric data were analyzed using Kruskal Wallis one way ANOVA.

Results and Discussion

In vitro establishment of nodal explants

Use of 20% Clorox for 20 minutes produced the cultures with lowest contamination rate (10%) compared to other treatments. Highest contamination (60%) was observed with 15% Clorox for 15 minutes. Browning is the major problem with both elite lines of cinnamon explants due to the presence of high phenolic contents. Best culture establishment was observed in WPM medium compared to MS medium.

Effect of the stage of maturity and media supplements in control of browning

Maturity stage 6 (Figure 2) produced the lowest percentage of browning when compared to other maturity stages. Percentage of browning was 0% (Figure 1a and 1c) in cultures originated from maturity stage 6 in both culture media supplemented with activated charcoal and ascorbic acid. Results indicate that the control of the browning of explants is most likely to be of twofold: first the physiological stage of maturity of the explant seems to be an important factor to start with and then in the culture medium the ascorbic acid and activated charcoal is playing an important role. Therefore, collectively those three factors: stage of maturity, the absorbent (activated charcoal) and biochemical inhibition of the synthesis of phenolic substances (ascorbic acid) get the browning under control. This outcome is very encouraging as browning is a significant hindrance to the success of cinnamon tissue cultures.

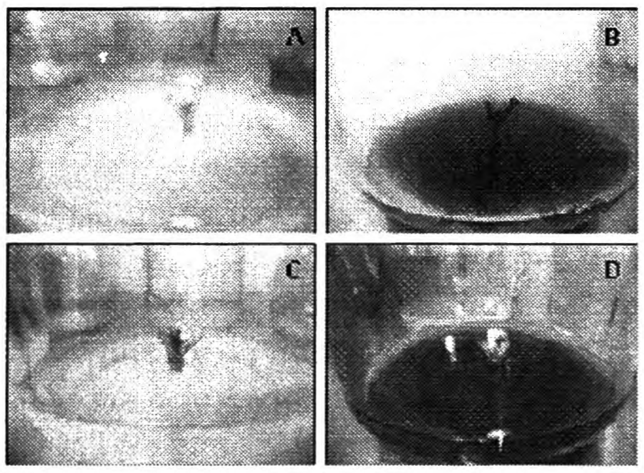

Figure 1: Established cultures; A - ascorbic acid in MS medium, B – activated charcoal in MS medium, C – ascorbic acid in WPM and D – activated charcoal in WPM

In all the other maturity stages 100% browning was observed even with the incorporation of absorbents and antioxidants (activated charcoal and ascorbic acid) in both WPM and MS media. We find this also as an important finding: missing the correct physiological stage of maturity at the time of obtaining the explants, irrespective of the fact that use absorbents and antioxidants in the medium still browning could occur to the detriment of culture establishment. Therefore, identification of the correct stage of maturity of explants seems to be the most important factor in controlling the browning in cinnamon tissue cultures.

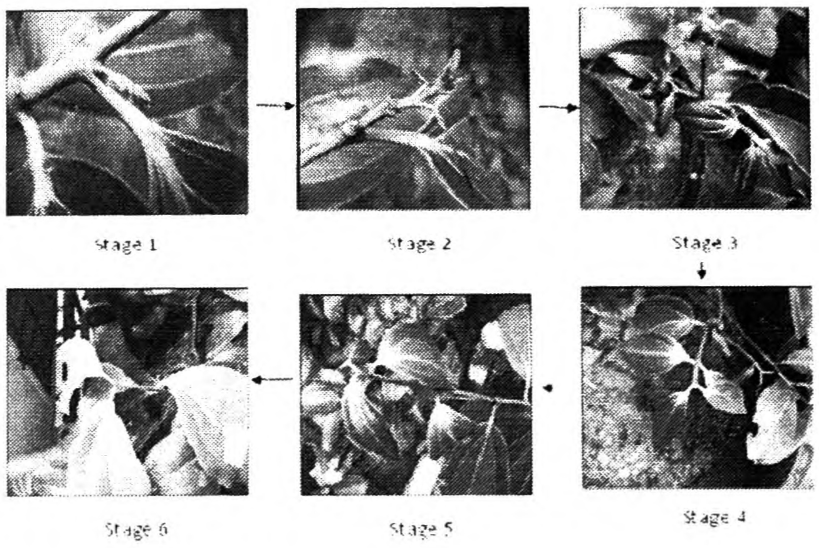

Figure 2: Different maturity stages used for the study

Accordingly, we conclude that the identification of correct maturity stage and incorporation of activated charcoal and ascorbic acid to the culture medium prevent browning of cinnamon nodal explant. Use of 20% Clorox for 20 minutes followed by ethanol for 30 seconds control the microbial contaminations of establishing cultures from field grown cinnamon plants. Regular spraying of fungicides, insecticides and fertilizer is important to minimize the fungal contaminations.

Acknowledgement

National Science Foundation in Sri Lanka is acknowledged for the financial assistance.

References

Krishnamoorthy B, Gopalam A, Abraham J 1992. Vriability and associated studies in cinnamon (*Cinnamomum verum*) J. Spices and Aromatic Crops. 1: 148-150.

PurseglovеJW 1977. Tropical Crops Dicotyledons: Longman, London. p.719.

Ranatunga J, Senanayake YM and Wijesekara ROB 2004.Cultivation and management of cinnamon. In *Cinammomum* and *Cassia.* The Genus *Cinnamomum* Ed: Ravindran PN , Nirmal BK, Shaylaja M, CRC Press. PP 121-129.

Sandigawad AM and Patil CG 2011. Genetic diversity in *Cinammomum zeylanicum* Blume. (Lauraceae) using random amplified polymorphic DNA (RAPD) markers. African J. of Biotechnology. 10 (19): 3682-3688.

Review on Potential for Keyhole Gardening Concept in Sri Lanka

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Abstract
In the context of limited resource availability, wastes can be valuable raw materials if they are recycled properly. However, improper waste management has lead to many problems in the present world. Considering the nutrient recycling, these wastes can be effectively used in home gardening. The keyhole gardens are the brainchild of humanitarian charities and missionaries for use in impoverished countries with poor soil, bad weather, and starving people. The Keyhole gardening is a concept which can overcome the waste associated problems in an effective manner. By using this method it is possible to promote waste recycling process, home gardening with low cost and minimum labor cost. This gardening process does not depend on the climatic effects, can manage the own water content, light condition and many other requirements. It is a method through which recycled materials are used to sustain a garden in otherwise unfavorable conditions. It is most common in developing countries where arable land is scarce, or arid climates that do not usually sustain typical garden lots. They are a form of raised bed gardening that nourish plant growth and utilize composting, recycling, and minimal water. They also provide protection from many animals and make tending easy and almost effortless. It can provide sustainable food sources for people seeking to step farther off the grid, conserve gardening resources, and provide an additional holistic avenue for recycling materials. This method needs to be popularized among the women in Sri Lanka to secure their wellbeing, health, economy and environment in their life. This is a sustainable waste disposal technique to utilize waste materials in home gardens in municipal and dry zones in Sri Lanka. This paper examines how keyhole garden concept can be used as a solution for sustainable waste handling in Sri Lanka.

Keywords: Composting, Keyhole gardening, Recycling, Sustainable, Waste
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Introduction

A keyhole garden is a sustainable garden practice, first used in Africa to grow in areas lacking in water and soil quality (Billstrom, 2015). It is a raised bed layers of lasagna-style: brown material (think cardboard boxes), then green material (grass clippings, etc.) then 0.15-0.24 m of growing soil, water thoroughly, then add plants (or seeds). Throughout the season appropriate table scraps and other organic compostable material are added to "feed" growing bed. In essence, keyhole gardens are a technique of gardening used to grow vegetables in dry climates and have been successfully used in places around the world that get far less water than even the driest places which first made popular in Africa and keyhole gardens are catching on in Texas and other hot, dry places. Keyhole gardens hold moisture and nutrients due to an active compost pile placed in the center of a round bed. Although most helpful in hot and dry locations a keyhole garden can improve growing conditions in other climates as well. Their low cost, low maintenance and versatility make them a desirable gardening option for yard and for gardening across the globe.

Every keyhole garden has a composting basket built into its center. This gives the garden a keyhole shape when viewed from above. The garden uses a number of layers to retain moisture and nourish the soil, making it more productive than a conventional garden. The composting basket replenishes the soil's nutrients as well. The garden is made from materials that are all available at low cost.

The center of manure and kitchen waste is the self-watering and self-fertilizing aspect that makes gardening life easier, reduces grocery bills, reduces the amount of water to the plants, eliminates fertilizing, and positively influences eating habits with fresh fruits and vegetables. Keyhole garden beds are commonly seen in permaculture gardens (Nikki, 2015). The concept is ideal for small spaces and can accommodate a variety of plants like vegetables, herbs, flowers, *etc.* In addition, permaculture Keyhole gardening can be easily adapted to fit the individual needs of the gardener. This review discusses the suitability and the potential about keyhole gardening concept in Sri Lankan condition.

Benefits of the keyhole garden

The keyhole garden helps to improve the enrichment of the soil which has several layers of organic materials in the garden and those are decomposed over time and add nutrients to the soil. The center compost basket provides a steady supply of plant nutrients and amendments and it is continuously replenishing the soil. Water is the limiting factor in dry zone season and zones. The keyhole gardening has ability to retain moisture. The garden requires less water to remain moist due to the layers are soaking up moisture and it uses less water with mulching and moisture from center basket. The stones of the keyhole garden wall absorb heat from the sun, protecting crops from the any climatic conditions. It uses a labor saving technology. The soil re-nourishment and moisture retention reduces the amount of time required to maintain the garden. Moreover, the garden shape makes it more accessible to sick or elderly gardeners. It is a low-cost design. All construction materials are readily available (at no cost) to gardeners. Only the gardeners might need to purchase is seeds for planting. It gives year-round vegetable production. Keyhole garden can be modified per owner's needs, like child or handicap accessibility. It requires a small area, a maximum of two meters (m) diameter (Walker, 2012) making gardening concept be practicable in urban areas. Inexpensive recycled material can use to build the garden, such as compost, kitchen scraps, manure and *etc.* The raised design removes threat of trampling by pets and humans. Keyhole gardens can be made temporary or permanent. Generally, common compost preparation methods required turnings more than two times for aeration. But in this concept, which saves steps by adding garden waste to the handy center compost basket. It is one of the environmental friendly concepts which can be an attractive feature with a good aesthetic design plan for developing countries like Sri Lanka. Waste become the major problem in Sri Lankan urban areas, this can be a kind of good solution for the garbage problem in the country.

How to make a Keyhole garden?

In a permaculture Keyhole garden, plants that are used on a regular basis (and those that require the most upkeep) are placed nearest the home quick and easy access. By using creative patterns and designs, gardeners can increase productivity, especially with the use of Keyhole garden beds. These beds can be designed in a number of ways, depending on the gardener's needs and preferences.

Following are the steps to make the Keyhole garden;

Step one is to prepare a dig over a space three square meters. Then, tie a stick to each end of 1.2m of garden twine. Place one end in the center of space and use the other to mark the circle in the ground. Then draw out an entrance triangle, from the circle to center, starting at width of 0.6 m. Secondly, lay down the canes 0.05- 0.1 m apart. Wrap wire around each one, attaching it until they are all in a line, do this at the top and the bottom of the canes. The total length should be about 1.3 m. It makes this into a cylinder and push into the ground at the center of space. Then half-fill this 'compost bucket' with top-soil to make a mound and line the inside of it with straw. Third step is laying stones, bricks or logs around the perimeter of garden; this could be a single layer or more, but enough to keep the soil in and build the stones higher at the entrance triangle. Start to make the first layer of the garden from broken plant pots or cans to improve drainage. Then start the filling the garden with the soil and compost in fourth step (make sure that the best soil goes top). The keyhole garden keeps piling up the soil until have a mound which slopes away from the basket. Finally, let the garden settle a week before planting seeds and seedlings. It wants to start with water the soil surface until the roots grow, then water the basket with waste or rain water, using the entrance. It is added already composted material to the basket and continues to add compostable food-waste. A circle or carpet over the top of the basket will help speed up the composting process. The compost will permeate the garden and give nutrients to the crops.

Planting in keyhole garden

The space, soil nutrients, and pest management are key considerations in the planning of garden. Companion planting is planting different kinds of crops together in the same garden in order to best satisfy those needs. Different methods include planting leafy crops next to root vegetables or planting pest-resistant vegetables (like onion or garlic) next to regular crops. There should be minimum four vegetables types are best to ensure that the garden will stay fertile and resist pests.

Preferred Crops for Keyhole Gardens

Suitable root crops are Carrot (*Daucuscarota*), Onion (*Allium cepa*), Beetroot (*Beta vulgaris*), Radish (*Raphanus sativus*), Mustard spinach (*Brassica rapa* var. *perviridis)*, Sweet

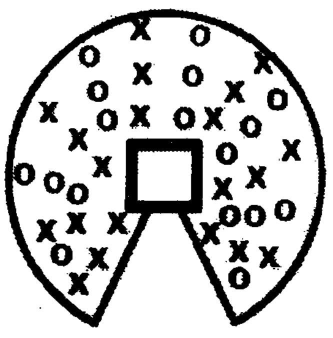

Figure 1: Example for companion planting in the Keyhole garden

potato(*Ipomea batata).* Vegitable crops are Cabbage (*Brassica oleracea*), Brinjal (*Solanum melongena*), Okra (*Abelmoschus esculentus*), Chilli (*Capsicum annuum*) and *etc.* Leafy crops are Spinach (*Spinaciaoleracea),* Swiss chard (*Beta vulgaris* subsp. *Vulgaris),* Lettuce (*Lactuca sativa),* KanKung (*Ipomoea aquatica*), Gotukola (*Centella asiatica*), Minchi (*Mentha spicata*), Koththamalli (*Coriandrum sativum*), Rathu thampala (*Amaranthus cruentus*)and *etc.*

Maintenance of the Keyhole garden

The garden should be watered regularly so that the garden soil is moist. Water from washing hands, laundry, or dishes are poured into the basket. The thatch and the composting in the basket will clean the water. Dry manure and topsoil should be replenished in the garden so that it does not become sapped of its fertility. Uncooked vegetable scraps, dry manure, eggshells, and compost are added to the basket. These replenish the soil. The basket will decompose within 1 or 2 years and should be replaced. The garden wall near the basket can be pulled away, allowing gardeners to remove the old basket and replace it. Over time, the garden may lose its nutrients, and vegetables stop growing well. Then it needs to rebuild the garden. This is usually done every 4–5 years.

Suitability of Keyhole garden concept to Sri Lanka

Daily waste collection by Local Authorities is estimated at 2,500tons (Abeygunawardana, 2009). It is important to recognize the basics and the complexity of the waste management process before attempting to find a sustainable and lasting solution to the problem in Sri Lanka.

In general people do not seem much aware of the (possible) environmental problems caused by the disposal of household waste. Due to lack of resources, solid waste is only collected frequently along main roads. Keyhole gardening concept is suitable for urban areas like Colombo, Kandy, Gampaha and *etc* due to huge accumulation of home garbage, which helps to dispose in to compact spaces in urban landlords. It helps to make food and health security in Sri Lanka. It gives an aesthetic value for the householders. This is a profitable way to spend unemployed women and housewives in Sri Lanka for their leisure time. Key hole gardening concept can be put into regulations and acts by Central Environment Authority and the Ministry of Forestry and Environment to reduce the garbage problem and activate as soon as possible via awareness programs of poster campaigns, advertisements and commercials, some education at schools and workshops.

Conclusion

The Keyhole garden concept is a sustainable waste disposal technique to utilize waste materials in home gardens in municipal and dry zones in Sri Lanka.

Reference

Abeygunawardana A 2009. Waste Management-Issues and Solutions [Online] http://efsl.lk/.../30%20%20Waste%20Management-%20issues%20and%20solu

Billstrom B 2015. Keyhole Gardening in Cold-Climates, [Online] https://morethanoregano.com/keyhole-gardening-in-cold-climates/.(Accessedon 22.08.2015).

Nikki P 2015. Keyhole Garden Beds–How to Make a Keyhole Garden [Online] http://www.gardeningknowhow.com/special/spaces/keyhole-garden-beds.htm. (Accessed on 29.08.2015).

Walker B 2012. Keyhole Gardens: A Drought Tolerant Composting Garden [Online]http://davesgarden.com/guides/articles/view/3726/. (Accessed on 01.09.2015).